# Redox cycling of geldanamycin produces superoxide radical: implications for studies of hsp90 and eNOS

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Geldanamycin is widely used as an inhibitor of heat shock protein 90 (HSP90). Reports that treatment of endothelial cells with geldanamycin inhibited HSP90 and impaired NO-dependent vasodilatation were used to support the role of HSP90 in function of endothelial NO synthase (eNOS). We now report that geldanamycin does generate superoxide radical during enzymatic and non-enzymatic redox cycling. Geldanamycin caused pronounced generation of superoxide radical in endothelial cells assessed by intracellular dihydroethidine (DHE) oxidation, which was not inhibited with L-NAME. Treatment of endothelial and smooth muscle cells with geldanamycin strongly increased superoxide radical formation detected with spin trap DEPMPO using ESR spectroscopy. Geldanamycin-induced formation of superoxide radical in endothelial cells was not inhibited by L-NAME suggesting insignificant role of eNOS in redox cycling of geldanamycin. Redox cycling of geldanamycin has been demonstrated using model flavin enzyme NADPH cytochrome P-450 reductase by detection of both semiquinone of geldanamycin and generation of superoxide radical. Flavin-containing enzymes are most likely to be involved in redox cycling of geldanamycin because DPI reduced superoxide production both in endothelial and smooth muscle cells. It was found that geldanamycin is a strong oxidant, which can directly oxidize ascorbate while reduced geldanamycin will increase superoxide radical formation. Non-enzymatic Redox cycling of geldanamycin was shown by the oxidation of cyclic hydroxylamine CPH, which was 50% inhibited by SOD, which support formation of superoxide radical by the reduced geldanamycin. It was found that geldanamycin caused 2-fold decrease in the level of NO generated by 3,4-dihydrodiazete 1,2-dioxide in smooth muscle cells.

The data obtained allow us to conclude that generation of superoxide by enzymatic and non-enzymatic redox cycling of geldanamycin is responsible for impaired NO-dependent vasodilatation. Geldanamycin is not specific inhibitor of HSP90 and because it generates superoxide radical, it is inapplicable as inhibitor of HSP90.